APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **13**, 63–68 (1999)

Heterobimetallic Complexes of Platinum(II) with Diferrocenylphenylphosphine and their *in Vitro* Activity Against P388 Leukaemia

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Four platinum(II) complexes of the general formula *cis*-[Pt{(Ferr)$_2$PhP}(DMSO)X$_2$], where X$_2$ = Cl$_2$, C$_2$O$_4$, O$_2$(CO)$_2$(C$_6$H$_{11}$)$_2$ and O$_2$(CO)$_2$CCH$_2$CH$_2$CH$_2$, have been synthesized and characterized physicochemically and spectroscopically as the first heterobimetallic platinum(II) complexes with the ligand diferrocenylphenylphosphine (Ferr = ferrocenyl). These complexes were tested *in vitro* against leukaemia cell line P388 using the MTT assay. The results obtained were compared with those of cisplatin, carboplatin, oxaliplatin and 5-fluorouracil.

Keywords: platinum(II); complexes; diferrocenylphenylphosphine; DMSO; leukaemia

Received 5 January 1998; accepted 21 April 1998

INTRODUCTION

Since the Rosenberg's discovery[1] that *cis*-dichlorodiammine platinum(II) complex, i.e. *cis*-[Pt(NH$_3$)$_2$Cl$_2$] (cisplatin) has potent antitumour activity, several amino–platinum complexes (Figure 1) were prepared and examined for their antitumour activity. Hence, carboplatin, [Pt(NH$_3$)$_2$\{O$_2$(CO)$_2$CCH$_2$CH$_2$CH$_2$\}], and oxaliplatin, [Pt(DACH)C$_2$O$_4$] or oxalato (1*R*,2*R*-cyclohexamediamine) platinum(II), have been discovered[2,3] and are used as drugs against certain types of tumours.

Other platinum complexes with various neutral amino ligands have been synthesized and tested for their antitumour activity with mixed success; they have been covered by at least two review articles[4,5] and numerous patents, e.g. Refs[6–8].

Complexes with neutral ligands other than nitrogen containing ones, e.g. phosphine, have also been synthesized and tested for their antitumour activity. The metallophosphine 1,1-bis(diphenylphosphino)ferrocene has been widely used as a mono- and di-phosphino ligand in the synthesis of heteropolymetallic complexes with gold,[9–11] platinum[12,13] and other metals.[14] Complexes with diferrocenylphenylphosphine, (Ferr)$_2$PhP, were uncommon and as far as the literature is concerned no platinum complexes with this ligand have been reported. We therefore attempted to prepare some platinum(II) complexes of this ligand of the general formula *cis*-[Pt{(Ferr)$_2$PhP}(DMSO)X$_2$] (X$_2$ = Cl$_2$, C$_2$O$_4$, O$_2$(CO)$_2$(C$_6$H$_{11}$)$_2$ and O$_2$(CO)$_2$CCH$_2$CH$_2$CH$_2$) and to examine the antitumour activity of these complexes against leukaemia cell line *P*388. Preliminary work was presented, in part, at conference[15] and elsewhere.[16–18]

EXPERIMENTAL

The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded at Jordan University, Amman, Jordan, on a Bruker 300 MHz spectrometer, using CDCl$_3$ as a solvent with tetramethylsilane (TMS) as an internal standard for ^{1}H and ^{13}C NMR and trimethyl phosphine (TMP) as an external standard for ^{31}P.

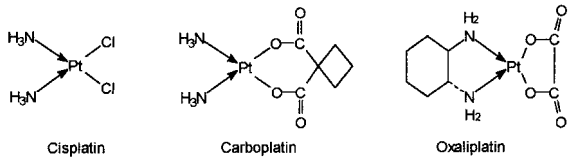

Figure 1 Amino–platinum complexes with antitumour activity.

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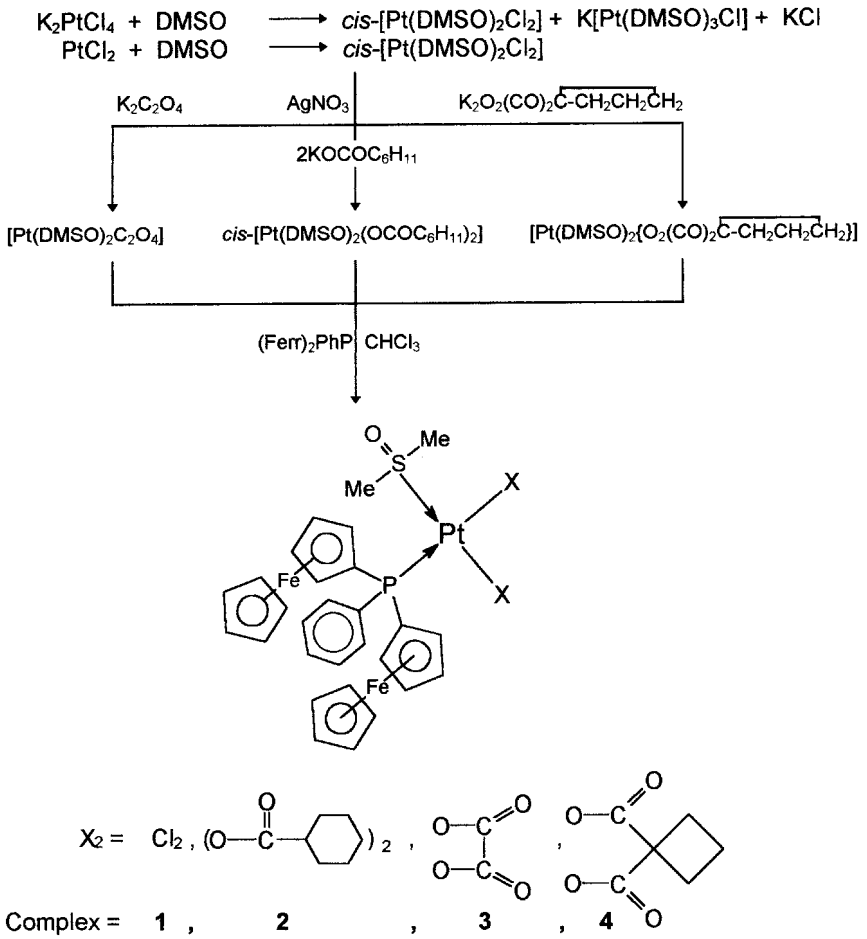


Scheme 1 The preperative route for complexes **1–4**.

IR spectra were recorded on a Nicolet (Impact 400) FTIR spectrometer in the range 4000–400 cm^{-1} using KBr discs. Elemental analyses of the complexes were performed at the University of Ulm, Germany.

Starting materials

The compounds $PtCl_2$, K_2PtCl_4, $H_2C_2O_4{\cdot}2H_2O$, $C_6H_{11}COOH$ and $CH_2CH_2CH_2C(COOH)_2$ were commercial products (Fluka) and were used without further purification. The compounds *cis*-$[Pt(DMSO)_2Cl_2]$, $[Pt(DMSO)_2(C_2O_4)]$, *cis*-$[Pt(DMSO)_2(C_6H_{11}COO)_2]$ and *cis*-$[Pt(DMSO)_2\{O_2(CO)_2C\text{-}CH_2CH_2CH_2\}]$ (DMSO-dimethyl sulphoxide) were prepared as described in our previous work.[19]

The complexes *cis*-$[Pt\{(Ferr)_2PhP\}(DMSO)X_2]$ were prepared according to Scheme 1.

Preparation of *cis*-$[Pt\{(Ferr)_2PhP\}(DMSO)X_2]$

Each complex *cis*-$[Pt(DMSO)_2X_2]$ ($X_2 = Cl_2$, C_2O_4, $O_2(CO)_2(C_6H_{11})_2$ and $O_2(CO)_2CCH_2CH_2CH_2$) (5 mmol) was suspended in chloroform (40 mL), the ligand $(Ferr)_2PhP$ (5.02 mmol) was added and the mixture was stirred vigorously under reflux for *ca* 60 min until dissolution was complete. The reaction mixture was filtered through silica gel, the clear orange solution thus obtained was reduced in volume to *ca* 10 ml and *n*-hexane was added to the point of turbidity. The mixture was left in the refrigerator overnight and the yellow–orange

Table 1 Physical properties of complexes **1–4**

Complex	Yield (%)	M.p. (°C)	Formula	Analysis (%) Found (Calcd) C	H	Selected IR bands (cm^{-1})[a] ν(S = O)	ν(C = O)
1	95	184–190 (dec)	$C_{28}H_{29}Cl_2PSOFe_2Pt$	40.9(40.7)	3.5 (3.7)	1139 s	
2	88	170–180 (dec)	$C_{42}H_{51}O_5PSFe_2Pt$	49.9(50.2)	5.0 (5.1)	1145 s	1627 m
3	76	210–230 (dec)	$C_{30}H_{29}O_5PSFe_2Pt$	42.8(42.9)	3.6 (3.5)	1140 s	1706 s, 1675 s
4	88	212–220 (dec)	$C_{34}H_{35}O_5PSFe_2Pt$	45.2(45.7)	4.2 (3.9)	1145 s	1660 s, 1643 s

[a] For IR bands; S, strong; m, medium.

Table 2 ^{1}H and ^{31}P NMR data,[a], δ (ppm) and J (Hz) of complexes **1–4**

	^{1}H NMR					^{31}P NMR
Complex	δ(CH_3/DMSO) [^3J(Pt-H)]	δ(C_5H_5)	δ(CH(2′,3′)) (CH(4′,5′))	δ(C_6H_5)	δ(Others)	J(Pt–P)
1	3.5 s (6H) [21.5]	4.3 s (10H)	4.5 dsep (4H) 4.6 dsep (4H)	7.3–7.5 m		3768.5
2	3.2 s (6H) [20.5]	4.3 s (10H)	4.6 dsep (4H) 4.6 dsep (4H)	7.4–7.9 m	1.1–1.9 m (C_6H_{11})	3907
3	3.4 s (6H) [21.6]	4.4 s (10H)	4.3 dsep (4H) 4.5 dsep (4H)	7.5–7.7 m		3898.5
4	3.3 s (6H) [20.6]	4.4 s (10H)	4.3 dsep (4H) 4.6 dsep (4H)	7.5–7.8 m	2.0 q (2H) (CH_2) 2.9 dq (4H) (CH_2)$_2$	3933

[a] Downfield from internal TMS for ^{1}H and from external TMP for ^{31}P NMR, using $CDCl_3$ as solvent; s, singlet; m, multiplet; q, quintet; dq, doublet of quintets; dsep, doublet of septets.

crystals thus formed were filtered, washed several times with *n*-hexane and dried under vacuum at *ca* 80 °C for several hours.

Biological work

Complexes

The four complexes 1–4 (Scheme 1) were dissolved in 10% DMSO. Serial dilutions of 0.1, 1.0 and 10.0 μg mL^{-1} were used and Millipore (0.2 μm) filtered under laminar flow conditions. Reference standards [cisplatin,carboplatin and 5-fluorouracil (5-FU)] were purchased from Bristol Myers Squibb (USA) and oxaliplatin was prepared, characterized and purified (HPLC) in our laboratories.

Cell lines

*P*388 cells were kindly supplied by Dr S. Tsukagoshi of the Japan Foundation for Cancer Research. They were maintained in RPMI-1640 medium (Nissui Pharmaceutical Co. Ltd) and supplemented with 5% foetal calf serum (Mitsubishi Chemical Industry Co. Ltd) and kanamycin (100 μg mL^{-1}).

Cytotoxicity assays

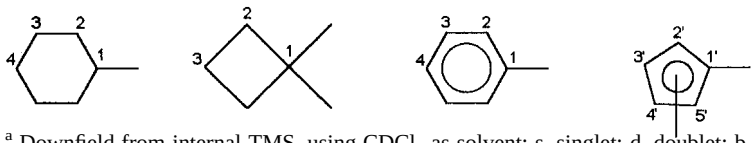
The cells (3×10^3 cells/well) were cultured in Corning disposable 96-well plates containing 100 μl of growth medium per well and were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 μl) were added to the cultures at day 1 after the transplantation. At day 3, 20 μl of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide solution (5 mg ml^{-1}) per well was added to each cultured medium. After a further 4 h of incubation, 100 ml of 10% sodium dodecylsulphat–0.01 M HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. Absorbance was measured with a microplate reader (Tohso MPR-A4i) with a two-wavelength system (550 and 700 nm). In all these experiments, three replicate wells were used to determine each point.

RESULTS AND DISCUSSION

The physical properties of complexes **1–4** are listed in Table 1 and their NMR data are compiled in

Table 3 ^{13}C NMR data,[a] δ (ppm) and J (Hz) of free $(Ferr)_2PhP$ and complexes **1–4**

		Ferrocene assignments						Phenyl assignments				Carboxylate assignments				
Compound	DMSO $\delta(CH_3)$[b]	$\delta(C_5H_5)$	$\delta(C_1')$ [J]	$\delta(C_2')$ [J]	$\delta(C_3')$ [J]	$\delta(C_4')$ [J]	$\delta(C_5')$ [J]	$\delta(C_1)$ [J]	$\delta(C_2)$ [J]	$\delta(C_3)$ [J]	$\delta(C_4)$ [J]	$\delta(C_1)$	$\delta(C_2)$	$\delta(C_3)$	$\delta(C_4)$	δ(CO)
$(Ferr)_2PhP$		69.1 s	77.9 d	72.0 d	69.9 d	70.5 d	72.7 d	39.2 d	133.9 d	127.9 d	128.8 b					
			[4.5]	[12.3]	[3.9]	[3.2]	[15.7]	[8.7]	21	[7.6]	—					
1	46.5 s	70.5 s	— [c]	74.6 d	70.7 d	71.8	74.8 d	— [d]	131.7 d	127.7 d	130.9 b					
				[18.9]	[9.8]	[8.3]	[16.7]		[11.3]	[11.6]	—					
2	45.0 s	70.5 s	— [c]	73.3 d	71.1 d	71.1 d	74.5 d	— [d]	131.9 d	127.7 d	130.7 d	46.5 b	29.9 s	25.9 s	26.1 s	180.4 s
				[10.9]	[9.1]	[9.1]	[13.0]		[11.5]	[11.7]	[2.9]		30.2 s	26.0 s	26.2 s	
3	45.0 s	70.4 s	— [c]	73.3 d	71.2 d	72.5 d	74.8 d	— [d]	132.0 d	128.2 d	130.8 b					165.1 s
				[12.5]	[9.5]	[8.6]	[11.0]		[11.3]	[11.7]	—					
4	44.0 s	70.3 s	— [c]	73.8 d	71.0 d	72.3 d	74.4 d	— [d]	132.0 d	128.0 d	131.5 d	55.0 s	31.0 s	16.0 s		176.3 s
				[12.4]	[9.5]	[8.2]	[10.1]		[11.2]	[12.4]	[2.3]					



[a] Downfield from internal TMS, using $CDCl_3$ as solvent; s, singlet; d, doublet; b, broad signal.
[b] The ^{195}Pt–^{13}C coupling constants could not be obtained due to insufficient signal-to-noise ratio.
[c] Signal obscured by the signals of $CHCl_3$.
[d] Signal obscured by noise.

Table 4 Cytotoxic activity against the P388 cell line of complexes **1–4**, with references

Compound	IC_{50} (μ g mL^{-1})
1	0.21
2	5.0
3	1.8
4	0.23
Cisplatin	0.17
Carboplatin	> 10
Oxaliplatin	> 10
5-FU	0.15

Tables 2 and 3. The reaction of *cis*-[Pt(DMSO)$_2$X$_2$] with 1/mol of the ligand (Ferr)$_2$PhP affords complexes **1–4** in which the phosphine ligand displaces one DMSO molecule. The remaining DMSO molecule in these complexes was identified by its ν(S=O) IR absorption band, which appears clearly at *ca* 1140 cm^{-1}, indicating S-bonding with platinum.[20] Further support for this argument is the proton signal appearing in the ^{1}H NMR spectra at $\simeq$ 3 ppm and $J(^{195}\text{Pt–SC–}^1\text{H})$ 21 Hz, indicating the presence of coordinated DMSO in the complex with S-bonding.[21] Similarly, the ^{13}C NMR signal for coordinated DMSO which appears at $\delta \simeq$ 45 ppm gives additional support for the presence of a DMSO molecule in the coordination sphere of the complex.

The ^{1}H and ^{13}C NMR data (Tables 2 and 3) for the organic species of the complexes **1–4** are within the expected ranges of values with small differences. For example, the ^{13}C NMR data for carbon atoms of both the C_5H_4 ring (attached to phosphine) and the C_5H_5 ring (of the free ligand) (Table 3) show some downfield shift (*ca* 1.5 ppm) with higher C–P coupling constants upon coordination; this is clearly due to coordination of phosphine with platinum. It should be noted here that the ^{13}C NMR data (Table 3) of the cyclohexane part of complex **2** shows an unusual feature, in the ^{13}C NMR spectrum reveals the presence of two different cyclohexyl groups (two signals for each of C2,C3,C4 and a broad one for C1). This does not mean that a mixture of two complexes together is present in the solid state rather than one complex with the *cis*-configuration, since all the spectroscopic data indicate the presence of one isomer. It is more likely that both the cyclohexyl groups of complex **2** have two different stereo geometries, i.e. non-symmetrical groups. The ^{13}P{^{1}H} NMR spectra of all the complexes (**1–4**) reveal the presence of one simple triplet of intensities *ca* 1:4:1 with $J(^{195}\text{P–}^{31}\text{P})$ values ranging between 3768.5 and 3933 Hz. This means that, in all cases, a single component is present in solution and also that the large *J* value (above 2500 Hz) assigning clearly to a *cis* isomer, rather than a *trans* isomer, is present; [14] the two electronegative groups Cl^- (complex **1**) or O^- (complexes **2–4**) are in a *cis* relationship to each other and in a *trans* relationship to both DMSO and (Ferr)$_2$ PhP ligands.

It should be noted that many unsuccessful attempts were made to react complexes **1–4** with nitrogen-containing ligands, e.g. pyridine, cyclohexylamine, 3,5-dimethylaniline, 3,5-dimethylpyrazole and the alkaloids harmaline or harmine. The aim of these attempts is to obtain new complexes by replacing the remaining DMSO molecule of complexes **1–4** by nitrogenous ligands.[16,17,18] It seems likely that the bulky ligand (Ferr)$_2$PhP does not allow extra accommodation for these nitrogenous ligands to coordinate with platinum in place of the smaller DMSO ligand.

Cytotoxicity evaluation

The cytotoxic activity *in vitro* of complexes **1–4** was evaluated against the leukaemic cell line P388 using the (MTT) assay. The results are summarized in Table 4, with values for the reference standards used in the present study: cisplatin, carboplatin, oxaliplatin and 5-fluorouracil (5-FU). As Table 4 shows, **1–4** all exhibited cytotoxic activity against the leukaemic cell line, but complexes **1** and **4** possess an excellent activity when compared with complexes **2** and **3**. The IC_{50} values of complexes **1** and **4**, i.e. 0.21 and 0.23 μg mL^{-1}, respectively display activity more than three orders of magnitude stronger than carboplatin and oxaliplatin. On the other hand, they showed more or less similar activity to those of cisplatin and 5-FU. These results are therefore very promising and further studies are in progress in our laboratories to test these complexes against other cell lines, such as A549 (lung adenocarcinoma), SW948 (colon carcinoma), KB (oval epidermoid carcinoma) and T47D (breast carcinoma).

Acknowledgments The authors are very grateful to Dr H. Itokawa (Professor of Natural Medicines), Tokyo University of Pharmacy and Life Science, Tokyo 192-03, Japan, for his kind help in performing the bioassay of complexes **1–4.**

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